

Cross sectional evaluation of biochemical markers of bone, cartilage, and synovial tissue metabolism in patients with knee osteoarthritis: relations with disease activity and joint damage

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Abstract

Objective—To analyse the relations between the urinary levels of type II collagen C-telopeptide (CTX-II) and glucosyl-galactosyl pyridinoline (Glc-Gal-PYD)—two newly developed biochemical markers of type II collagen and synovial tissue destruction respectively—disease activity and the severity of joint destruction in patients with knee osteoarthritis (OA). The clinical performance of these two new markers was compared with that of a panel of other established biochemical markers of connective tissue metabolism. **Methods**—The following biochemical markers were measured in a group of 67 patients with knee OA (mean age 64 years, median disease duration eight years) and in 67 healthy controls: for bone, serum osteocalcin, serum and urinary C-telopeptide of type I collagen (CTX-I); for cartilage, urinary CTX-II, serum cartilage oligomeric matrix protein (COMP), and serum human cartilage glycoprotein 39 (YKL-40); for synovium, urinary Glc-Gal-PYD, serum type III collagen N-propeptide (PIIINP), serum hyaluronic acid (HA); and for inflammation, serum C reactive protein. Biochemical markers were correlated with pain and physical function (WOMAC index) and with quantitative radiographic evaluation of the joint space using the posteroanterior view of the knees flexed at 30°.

Results—All bone turnover markers were decreased in patients with knee OA compared with controls (–36%, –38%, and –52%, $p < 0.0001$ for serum osteocalcin, serum CTX-I and urinary CTX-I, respectively). Serum COMP (+16%, $p = 0.0004$), urinary CTX-II (+25%, $p = 0.0009$), urinary Glc-Gal-PYD (+18%, $p = 0.028$), serum PIIINP (+33%, $p < 0.0001$), and serum HA (+233%, $p < 0.0001$) were increased. By univariate analyses, increased urinary Glc-Gal-PYD ($r = 0.41$, $p = 0.002$) and decreased serum osteocalcin ($r = -0.30$, $p = 0.025$) were associated with a higher total WOMAC index. Increased urinary CTX-II ($r = -0.40$, $p = 0.0002$) and Glc-Gal-PYD ($r = -0.30$, $p = 0.0046$) and serum PIIINP ($r = -0.29$, $p = 0.0034$) were the only markers which correlated with joint surface area. By multivariate analyses, urinary Glc-Gal-PYD and CTX-II

were the most important predictors of the WOMAC index and joint damage, respectively.

Conclusion—Knee OA appears to be characterised by a systemic decrease of bone turnover and increased cartilage and synovial tissue turnover. CTX-II, Glc-Gal-PYD, and PIIINP may be useful markers of disease severity in patients with knee OA.

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Osteoarthritis (OA), the most common joint disease, is not only characterised by cartilage destruction but also by alteration of bone and synovial tissue metabolism, though their relative importance in the initiation and progression of OA is still debated. The most established method for assessing joint damage in OA is joint space width (JSW) measurement using plain x rays. However, when radiological diagnosis is established, significant joint damage has often already occurred. In addition, because changes of JSW are small compared with the precision error of x rays, at least one year and preferably two years are usually necessary to assess accurately the progression of joint damage or its reduction by treatment.¹ Clearly, to identify patients with a high risk for destructive OA and to monitor drug efficacy, more sensitive techniques than plain x rays are needed. Magnetic resonance imaging is currently being optimised for this purpose; alternatively, specific and sensitive biochemical markers reflecting abnormalities of the turnover of bone, cartilage, and synovium tissues may be useful for the investigation and monitoring of OA.

Biochemical markers are molecules of connective tissue matrices which are released into biological fluid during the process of tissue turnover. Several biochemical markers of bone, cartilage, and synovium have been described in experimental animal models and in humans with OA (for a review see Garnero *et al*).² It has been suggested that these markers might be useful for identifying patients at high risk for progression and for assessing therapeutic response in OA because of their faster response compared with x rays.^{3–9} However, most previous studies investigating the value of biochemical markers have several limitations, which include (a) the study of only a small group, (b) the measurement of a single or few markers of either cartilage, bone, or synovium turnover—

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although the association of markers reflecting metabolism of these three tissues is likely to give a better picture of the pathophysiological pattern of OA—(c) the absence of the measurement of a marker which reflects specifically type II collagen degradation, a hallmark of OA,^{10–12} and (d) the lack of a quantitative measure of joint destruction.

Recently, immunoassay using antibodies recognising either a neo-epitope generated by collagenases in the triple helix domain of type II collagen¹³ or fragments of the telopeptides^{14,15} has been developed to monitor type II collagen degradation. Using an assay recognising type II collagen C-telopeptide fragments (CTX-II) in urine, we recently found increased levels in patients with active rheumatoid arthritis (RA) which correlated with the extent of joint destruction.¹⁵ Several markers have been suggested to assess synovitis in OA and RA. These, which include serum C reactive protein,¹⁶ hyaluronate (HA), and type III collagen N-terminal propeptide (PIINP), are not specific for synovial tissue.² We recently showed that glucosyl-galactosyl pyridinoline (Glc-Gal-PYD), a non-reducible cross link of collagen molecules, is present in human synovium tissue and is released during its destruction in vitro, but it is virtually absent from bone, cartilage, and other soft tissues.¹⁷ Preliminary in vivo data indicate increased U-Glc-Gal-PYD levels in patients with RA but not in patients with Paget's disease, which correlate with the extent of bone erosion assessed by x rays.

That study aimed at assessing the clinical performance of the levels of U-CTX-II and U-Glc-Gal-PYD and of a panel of existing biochemical markers of connective tissue metabolism in patients with knee OA.

Subjects and methods

PATIENTS WITH KNEE OSTEOARTHRITIS

The study included 67 outpatients with knee OA (41 women, 26 men; mean (SD) age 63.6 (9.1) years) who were attending the department of rheumatology of the Centre Hospitalier Lyon Sud (Lyon, France). All patients fulfilled the American College of Rheumatology criteria for primary knee OA.¹⁸ All patients had had chronic daily pain of the knee for at least three months (median eight years) and radiographic evidence of OA with joint space narrowing (JSN) when using the posteroanterior view of the knees flexed at 30° (Schuss view). Patients presenting with an advanced stage of OA, with a minimum JSW <1 mm, were excluded. All women were postmenopausal and none of the patients was receiving treatment that might interfere with bone metabolism, such as oestrogen replacement therapy, dilantin, thyroid replacement therapy, and diuretics. Pain and physical function were assessed by the Western Ontario and McMaster Universities multifunctional (WOMAC) index,¹⁹ using a visual analogue scale as a grading system. The WOMAC index comprises three subscales—namely, pain, stiffness, and physical function, which were analysed separately. We also calculated an aggregated score (total WOMAC score) by the summation of the

24 different items, which is the simplest and most commonly used approach to mix the three indices according to Bellamy *et al* (Womac Index User's Guide, London Ontario, 1995).

HEALTHY SUBJECTS

Healthy subjects included 38 postmenopausal women with a mean (SD) age of 63.2 (8.1) years (range 50–80) and 29 men aged 62 (8.2) (range 53–79). Menopausal status was defined as the absence of menses for at least 12 months. Healthy women and men were randomly selected from two large population based cohorts who were taking part in prospective studies on the determinants of bone loss in women (OFELY study) and men (MINOS study).^{20,21} The cohort of the OFELY study comprises 1039 healthy female volunteers, aged 31–89, randomly selected from affiliates of the section of a health insurance company (Mutuelle Générale de l'Education Nationale) from the Rhône district—that is, the same region of France as the patients with knee OA. The cohort of the MINOS study comprises 842 healthy male volunteers, aged 50–85, randomly selected from affiliates of the section of a health insurance company (Société de Secours Minière de Bourgogne) in Montceau les Mines, a town located in the same region as that of the healthy women and patients with knee OA. None of the healthy women and men had evidence of symptomatic OA as assessed by clinical examination of the hands performed by an experienced rheumatologist and by the answer to the following question: "Has a doctor ever told you that you had osteoarthritis". Subjects who answered "yes" to this question were then asked at what age the doctor diagnosed OA for the first time. Additionally, x ray films of the thoracic and lumbar spine were obtained in all subjects. Spine films were graded with a standard atlas to document the severity of disc degeneration and osteophyte formation using the method of Lane *et al*,²² with a grade of 0 = normal, 1 = mild narrowing or mild osteophytes, or both, 2 = moderate-severe (2–3) narrowing and or moderate-severe (2–3) osteophytes. Subjects who were grade 2 (moderate-severe) were excluded from the control group. All subjects were healthy without any disease or treatment that might interfere with bone or joint metabolism, including hormone replacement therapy in postmenopausal women.

RADIOGRAPHS AND MEASUREMENTS OF KNEE

JOINT SPACE PARAMETERS

Bilateral standing tibiofemoral radiographs were taken in a posteroanterior view with the patient flexing both knees at approximately 30° (Schuss).²³ The patellae touched the film cassette, the toes pointed straight ahead vertically relative to the knee, and the pelvis touched the table. The angle of knee flexion was measured for all patients with a goniometer and ranged from 28° to 35° (mean (SEM) 29.75 (1.57)). With the aid of fluoroscopy, the

Table 1 Biochemical markers of bone, cartilage, and synovium turnover in patients with knee osteoarthritis (OA) and controls. Results are shown as mean (standard deviation). All *p* values refer to logarithmic transformed data

Biochemical markers*	Knee OA (n=67)	Controls (n=67)	<i>p</i>	<i>p</i> adj. for BMI*
Bone markers				
S-total OC (ng/ml)	12.6 (6.8)	19.6 (6.9)	<0.0001	0.0013
U-CTX-I (mg/mmol Cr)	147 (78)	307 (191)	<0.0001	0.0002
S-CTX-I (nmol/l)	2.3 (1.3)	3.7 (1.5)	<0.0001	0.0004
Cartilage markers				
U-CTX-II (ng/mmol Cr)	431 (154)	345 (140)	0.0009	0.0009
S-COMP (ng/ml)	1677 (360)	1449 (190)	0.0004	0.0002
S-YKL-40 (ng/ml)	121 (155)	81 (49)	0.25	0.99
Synovium/inflammation markers				
U-Glc-Gal-PYD (nmol/mmol Cr)	5.2 (1.8)	4.4 (1.2)	0.028	0.028
S-PIIINP (mg/l)	4.21 (1.41)	3.16 (0.55)	<0.0001	0.04
S-HA (mg/l)	120 (129)	36 (16)	<0.0001	<0.0001
S-CRP (mg/l)	3.03 (3.5)	2.5 (4.3)	0.12	0.76

*BMI = body mass index; S = serum; U = urine; OC = osteocalcin; CTX-I, CTX-II = types I and II collagen C-telopeptide, respectively; COMP = cartilage oligomeric matrix protein; YKL-40 = human cartilage glycoprotein 39; Glc-Gal-PYD = glucosyl-galactosyl pyridinoline; PIIINP = type III collagen N-propeptide; HA = hyaluronic acid; CRP = C reactive protein.

x ray beam position was adjusted to obtain a horizontal plateau. All radiographs were measured with an image analysis computer (Mediscan, Hologic), as previously described,²³ by a single observer. A constant part of the femorotibial compartment was automatically determined from two peripheral points at a constant 172°. The joint space area (JSA) was the cross sectional area on the *x* ray film delineated on the margin of the femoral condyle and of the tibial plateau within the constant compartment automatically determined by the computer program. The interbone distance at the narrowest point (minimal JSW) was also measured by the computer. The intra-observer reproducibility was assessed by measuring 10 knee OA radiographs five times. For each radiograph a coefficient of variation (CV) based on the five measurements was obtained. The mean (SD) of the 10 individual CVs was 3.3 (1.4) % for JSA and 5.0 (1.8) % for minimal JSW.²³

BIOCHEMICAL MEASUREMENTS

Fasting blood samples and second morning void urine samples were obtained from all patients with OA on the day the radiographs were taken, and pain and function were assessed. The same types of sample were obtained from controls. All biological samples were kept frozen at -80°C until assayed.

Markers of bone turnover

Serum total osteocalcin (S-total OC), a specific marker of bone formation, was measured by a two site assay measuring both intact and N-mid-peptide using an automatic system (Kryptor-Osteo, CisBiointernational, Gif/Yvette, France). Measuring N-mid-peptide—the main proteolytic fragment of OC—allows correction for the potential degradation of OC in vitro and the determination of precise measurements.²⁴ Intra- and interassay variations (CV) are lower than 2.5% and 3% respectively. Urinary excretion of β isomerised C-terminal cross linking telopeptide of type I collagen (U-CTX-I) was measured by the Crosslaps enzyme linked immunosorbent assay (ELISA) (Osteometer Biotech, Herlev, Denmark).²⁵ This assay uses a polyclonal antiserum raised against the β isomerised EKAH β DGGR sequence of the C-telopeptide of $\alpha 1$ chains of human type I collagen. Intra- and interassay CVs are lower than 6% and 9%, respectively.²⁶ Serum β isomerised C-terminal cross linking telopeptide of type I collagen (S-CTX-I) was measured by an immunoassay (Serum Crosslaps one step, Osteometer Biotech, Herlev, Denmark). This serum resorption marker assay uses two monoclonal antibodies raised against a synthetic peptide with an amino acid sequence specific for a part of the C-telopeptide of the $\alpha 1$ chain of type I collagen (Glu-Lys-Ala-His- β Asp-Gly-Gly-Arg).²⁶ Intra- and interassay CVs are lower than 8%.²⁶

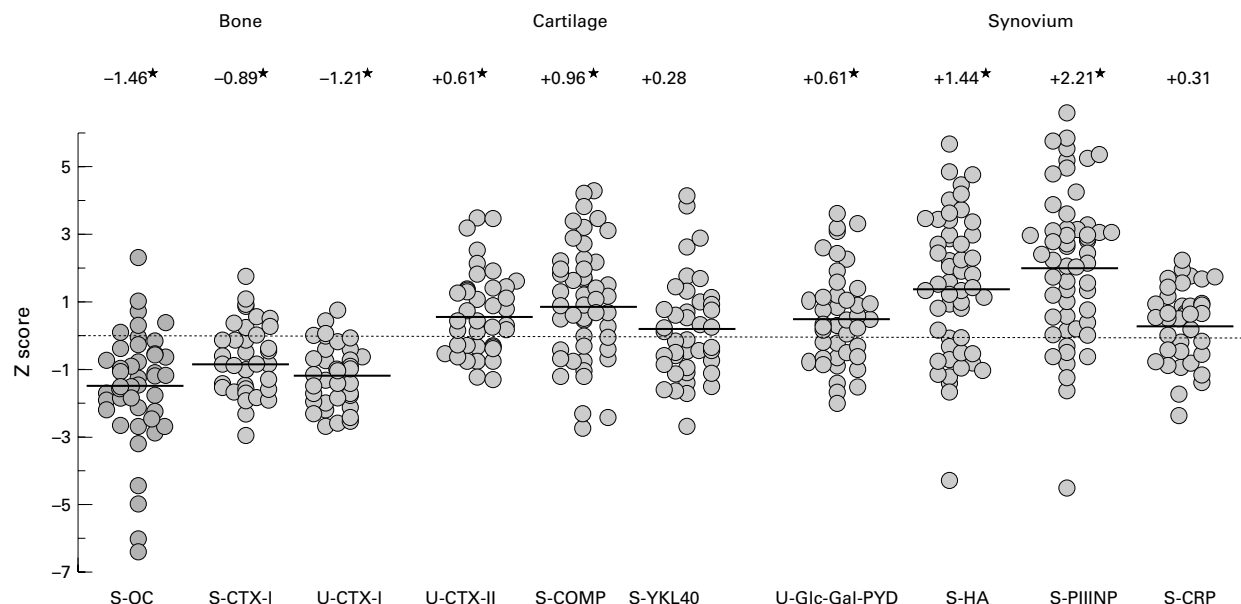


Figure 1 Individual values of biochemical markers of bone, cartilage, and synovium turnover in 67 patients with knee OA. Each value is expressed as a Z score—that is, as the number of standard deviations from the mean of 67 healthy controls matched for age. **p* < 0.0001 v 0. S = serum; U = urine; OC = osteocalcin; CTX-I, CTX-II = types I and II collagen C-telopeptide, respectively; COMP = cartilage oligomeric matrix protein; YKL-40 = human cartilage glycoprotein 39; Glc-Gal-PYD = glucosyl-galactosyl pyridinoline; HA = hyaluronic acid; PIIINP = type III collagen N-propeptide; CRP = C reactive protein.

Table 2 Correlation between biochemical markers of bone, cartilage, and synovium turnover and WOMAC indices in patients with knee osteoarthritis

Biochemical markers‡	Pain	Stiffness	Physical function	Total score†
Bone markers				
S-total OC	-0.24*	-0.19	-0.27*	-0.30*
U-CTX-I	0.06	-0.05	0.13	0.05
S-CTX-I	0.01	-0.10	0.01	-0.002
Cartilage markers				
U-CTX-II	0.06	0.09	0.2	0.18
S-COMP	0.05	0.02	-0.02	-0.009
S-YKL-40	0.16	0.17	0.06	0.094
Synovium/inflammation markers				
U-Glc-Gal-PYD	0.31*	0.24	0.41**	0.41**
S-PIIINP	0.29*	0.33*	0.22	0.25
S-HA	-0.12	-0.18	-0.07	-0.08
S-CRP	0.24	0.05	0.26*	0.23

* $p < 0.05$; ** $p < 0.005$.

†The total score is the sum of the of pain, stiffness, and physical function items.

‡For abbreviations, see table 1.

Markers of cartilage turnover

Urinary C-terminal cross linking telopeptide of type II collagen (U-CTX-II) was measured by an ELISA based on a monoclonal antibody raised against a linear six amino acid epitope of the type II collagen C telopeptide.¹⁵ Intra- and interassay CVs are lower than 8% and 10%, respectively.¹⁵ Serum cartilage oligomeric matrix protein (S-COMP) was measured by an ELISA using native human COMP as standard and immunogen (Wielisa COMP, Wieslab, Lund, Sweden). Intra- and interassay CVs are lower than 5%. Serum human cartilage glycoprotein 39 or YKL-40 was measured by a two site ELISA using antibodies raised against YKL-40 purified from supernatants of the MG63 human osteosarcoma cell line (Chondrex, Metra Biosystems, Mountain View, CA, USA).²⁷ Intra- and interassay CVs are lower than 4% and 6%, respectively.²⁷

Markers of synovitis and inflammation

Serum hyaluronan (S-HA) was measured by radioimmunoassay (RIA; Pharmacia HA test, Pharmacia and Upjohn Diagnostics AB, Uppsala, Sweden), based on the use of specific hyaluronic acid binding protein isolated from bovine cartilage. Intra- and interassay CVs are lower than 10%. Serum procollagen type III N-propeptide (S-PIIINP) was measured by

RIA based on a polyclonal antibody (PIIINP-RIA kit, Farmos Diagnostica, Oulunsala, Finland).²⁸ The assay detects the authentic propeptide and other larger related antigens, but is insensitive to the degradation products of the propeptide. Intra- and interassay CV (%) ranged from 6% to 8%.²⁸ Urinary glucosyl-galactosyl pyridinoline (U-Glc-Gal-PYD) was measured on non-hydrolysed samples by high performance liquid chromatography.¹⁷ Intra- and interassay variations are lower than 8% and 11%.¹⁷ Serum C reactive protein (S-CRP) was assayed using an ultrasensitive immunonephelometry method (N Latex CRP mono, Behringwerke AG, Marburg, Germany) on a BNA Behring nephelometer. The intra- and interassay variations are lower than 5% and the detection limit is of 0.2 mg/l.

STATISTICAL ANALYSES

Distribution analysis showed that biochemical markers were not normally distributed and thus were log transformed to obtain normal distribution before statistical analyses. Comparisons of marker levels between patients with knee OA and controls were assessed by Student's unpaired *t* test, before and after adjustment for body mass index (BMI) calculated as the ratio of weight (kg) and the square of height (m²). Correlation between markers and WOMAC indices was assessed by linear regression analyses. Relations between biochemical marker levels and radiological joint space parameters (JSA and minimal JSW) were assessed by a generalised estimating equation model using each knee (both right and left) as the observation unit, while accounting for the correlation between both knees of the same subject's knees.^{29,30} All statistical analyses were carried out using SAS (SAS Institute Inc, Cary, NC).³¹

Results

BONE, CARTILAGE, AND SYNOVIUM TURNOVER IN PATIENTS WITH KNEE OSTEOARTHRITIS

Patients with knee OA did not differ from controls for age, sex distribution, and height. However, and as expected, patients with knee OA were heavier (80.6 (13.5) *v* 68.5 (14.2) kg, $p < 0.0001$) and had a higher BMI (29.9 (5.1) *v* 26.2 (3.7) kg/m², $p < 0.0001$) than controls. In the whole population, total OC ($r = -0.39$, $p < 0.0001$), S-CTX-I ($r = -0.26$, $p = 0.009$), and U-CTX-I ($r = -0.39$, $p < 0.001$) correlated negatively with BMI, whereas S-YKL-40 ($r = 0.24$, $p = 0.015$), S-CRP ($r = 0.32$, $p = 0.005$), S-PIIINP ($r = 0.41$, $p < 0.0001$), and S-HA ($r = 0.33$, $p = 0.002$) correlated positively with BMI. No significant correlation between BMI and S-COMP, U-CTX-II, and U-Glc-Gal-PYD was found.

In patients with knee OA all markers of bone turnover were significantly decreased compared with controls both before and after adjustment for BMI (table 1, fig 1). Among markers of cartilage turnover, U-CTX-II and S-COMP were significantly increased, whereas no significant difference was observed for S-YKL-40. For synovium, U-Glc-Gal-PYD, S-PIIINP, and S-HA were significantly increased in patients with knee OA (table 1, fig

Table 3 Correlation between biochemical markers of bone, cartilage, and synovium turnover, and the WOMAC index with radiographic parameters of the knee joint space. Correlation coefficients and *p* values were obtained by generalised estimating equation using each knee (both right and left) as an observation unit (total of 134 knees), while accounting for the correlation between both knees of the same subject (see statistical analyses)

Biochemical markers*	Joint surface area		Minimal JSW	
	<i>r</i> Value	<i>p</i> Value	<i>r</i> Value	<i>p</i> Value
Bone markers				
S-total OC	-0.10	0.34	-0.10	0.30
U-CTX-I	-0.24	0.02	-0.17	0.09
S-CTX-I	-0.10	0.22	0.13	0.14
Cartilage markers				
U-CTX-II	-0.40	0.0002	-0.36	0.0017
S-COMP	-0.15	0.17	-0.13	0.23
S-YKL-40	-0.19	0.63	-0.19	0.06
Synovium markers				
U-Glc-Gal-PYD	-0.30	0.0046	-0.26	0.017
S-PIIINP	-0.29	0.0034	-0.31	0.006
S-HA	-0.18	0.25	-0.16	0.13
S-CRP	-0.11	0.27	-0.03	0.78
Total WOMAC index	0.10	0.28	0.08	0.43

*For abbreviations, see table 1.

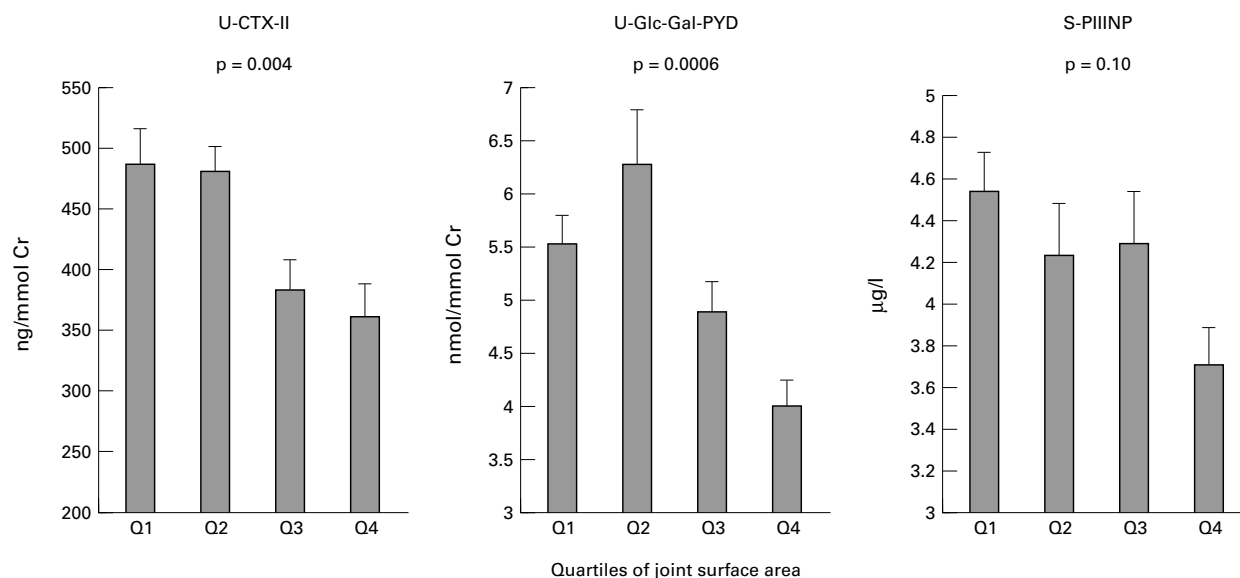


Figure 2 Levels of U-CTX-II, U-Glc-Gal-PYD, and S-PHIINP according to quartiles of joint space area (JSA) of the knee. A total of 134 knees were analysed. Bars represent the mean value (SEM) of the biochemical marker in each quartile of JSA. *p* Values apply to the comparison of trend in biochemical marker levels for increasing JSA, assessed by analysis of variance.

1). However, after adjustment for BMI, the differences decreased markedly for S-PHIINP. No significant difference between patients with knee OA and healthy controls was seen for S-CRP.

RELATIONS BETWEEN BIOCHEMICAL MARKERS OF BONE, CARTILAGE, AND SYNOVIUM TURNOVER AND SEVERITY OF KNEE OSTEOARTHRITIS

At the time of the evaluation, 35% of patients had a knee effusion, 45% woke in the night because of pain, and 12% had morning stiffness greater than 20 minutes. No significant difference in biochemical marker levels between patients with and without knee effusion was seen except for S-COMP (1812 (363) *v* 1591 (349) ng/ml, *p*=0.02 in patients with and without effusion, respectively) and for S-YKL-40 (151 (187) *v* 100 (129), *p*=0.026). Patients who woke in the night with pain had slightly higher CRP levels (3.7 (3.8) *v* 2.4 (3.1), *p*=0.03). No significant association was seen between morning stiffness and biochemical marker levels.

Tables 2 and 3 show the results of the univariate analyses relating the WOMAC indices and radiological parameters of joint destruction with levels of biochemical markers.

Increased levels of U-Glc-Gal-PYD (*p*=0.002) and decreased levels of S-total OC (*p*=0.025) were associated with an increased total WOMAC score (table 2). None of the other biochemical markers was predictive of the total WOMAC score. When the different components of the WOMAC index were analysed separately, results similar to those found for the total WOMAC score were obtained, though S-PHIINP correlated significantly with pain and stiffness (table 2).

Among the various biochemical markers, only U-CTX-II, U-Glc-Gal-PYD, and S-PHIINP correlated significantly with JSA and minimal JSW of the knee, increased levels being associated with increased JSN (table 3). When JSA was categorised in quartiles, decreased JSA was significantly associated with increased levels of U-CTX-II (*p*=0.004) and U-Glc-Gal-PYD (*p*=0.0006), but not with S-PHIINP (fig 2). Patients with JSA values in the lowest quartile had, on average, levels of U-CTX-II and U-Glc-Gal-PYD 35% and 38% higher, respectively, than women with JSA in the highest quartile (*p*<0.001). The total WOMAC index was not correlated with any of the radiographic joint space parameters (table 3).

Table 4 Multivariate analysis relating WOMAC index and radiographic parameters of joint destruction to biochemical markers of connective tissue metabolism in patients with knee osteoarthritis

Dependent variable	Predictors† (independent variables)	Coefficient (SE)	Standardised coefficient* (SE)	<i>p</i> Value
Total WOMAC	U-Glc-Gal-PYD	0.082 (0.027)	0.396 (0.129)	0.0036
	S-total OC	-0.016 (0.007)	-0.293 (0.129)	0.017
	<i>r</i> ² (%)		25	0.0011
Joint surface area	U-CTX-II	-0.622 (0.185)	-0.343 (0.102)	0.0012
	S-PHIINP	-0.463 (0.214)	-0.220 (0.102)	0.033
	<i>r</i> ² (%)		20	<0.0001
Minimal joint space width	U-CTX-II	-0.309 (0.105)	-0.304 (0.103)	0.004
	S-PHIINP	-0.277 (0.121)	-0.236 (0.103)	0.025
	<i>r</i> ² (%)		18	0.0003

*Standardised coefficients allow a direct comparison of the relative contribution of each predictor to the dependent variable.

†For abbreviations, see table 1.

To assess the relative contributions of the different biochemical markers in determining the total WOMAC index and radiological indices of joint destruction, multivariate models, including total WOMAC index, JSA, and minimal JSW as the dependent variables, and levels of biochemical markers as the independent variables, were used (table 4). U-Glc-Gal-PYD was the most important predictor of the total WOMAC index. After this parameter was included in the model, only S-total OC was negatively associated with the total WOMAC index. U-CTX-II was the most important biochemical marker in predicting JSA and minimal JSW. After this marker was included in the model, only S-PIINP was significantly associated with the radiological index of joint destruction, this combination explaining 20% and 18% of the interindividual variability of JSA and minimal JSW, respectively.

Discussion

In this study, using a panel of biochemical indices of bone, cartilage, and synovial tissue turnover, we found that the recently developed markers Glc-Gal-PYD and CTX-II, which reflect specifically the degradation of synovium and cartilage tissue, were the most predictive of the total WOMAC index and radiological parameters of joint destruction, respectively.

When investigating levels of bone markers measured in peripheral fluids it is difficult to analyse whether they reflect bone turnover abnormalities in the subchondral bone of affected joints or generalised skeletal alterations, or both, because both of them are likely to be modified in patients with OA. Osteoarthritis is indeed a process characterised by alteration of the osteoblastic phenotype,^{32,33} increased subchondral trabecular thickness, and bone sclerosis, which may be responsible for trabecular microfractures.³⁴ On the other hand, progressive OA is often characterised radiographically by bone destruction³⁵ and increased juxta-articular bone turnover as indicated by bone scintigraphy³⁶ and increased OC levels in the synovial fluid of affected joints.³⁷ At the skeletal level, however, OA is often associated with increased bone mineral density (BMD),³⁸ though this may not always translate into reduced fracture risk.^{39,40} Increased BMD might result, in part, from decreased bone loss, which has been reported in some,⁴⁰⁻⁴² but not all,⁴³ studies. Because in postmenopausal women low bone turnover is associated with a reduced rate of loss⁴⁴ and increased BMD,²⁰ decreased levels of markers of bone turnover should be expected in patients with OA. Actually, studies on bone turnover markers in OA have yielded conflicting data. For bone formation markers, decreased serum OC levels were found in a small group of elderly patients with OA of undefined origin,³⁷ whereas more recent data including 30 women with knee OA aged over 60 found no significant alteration of the level of this marker.⁴⁵ Interestingly, a recent longitudinal study performed in a large group of pre- and perimenopausal women reported decreased levels of serum OC in patients with

prevalent knee OA, and low levels were associated with an increased incidence of OA, suggesting that decreased bone formation is associated with a higher risk of developing knee OA.⁴² Decreased bone formation, as assessed by serum bone alkaline phosphatase, was also reported in postmenopausal women with spinal OA.⁴⁶ For bone resorption, most of the previous studies used the urinary excretion of total pyridinoline (PYD) and deoxypyridinoline (DPD). Increased PYD and DPD levels were reported in most,^{8,45,47-49} but not all,^{46,50} studies. However, because a high amount of PYD is present not only in bone but also in cartilage and synovium tissue,⁵¹ the origin and significance of their urinary levels in OA is unclear. In our study we used two recently developed markers reflecting specifically the degradation of bone type I collagen—thus precluding any contribution from cartilage—and found a 50–60% decreased bone resorption in patients with knee OA. Altogether, the bone marker data obtained in our study lend support to the view that decreased bone turnover at the skeletal level may, in part, be responsible for increased BMD in patients with OA.

In this study we confirm that levels of S-COMP, S-PIINP, and S-HA⁵²⁻⁵⁵ are higher in patients with knee OA than in age matched controls, indicating increased cartilage and synovial tissue turnover. In contrast, we could not confirm the higher YKL-40 levels which were reported in an isolated study to be increased in patients with late, but not with early, knee OA.²⁷ Thus the significance of S-YKL-40 in OA remains unclear. In patients with early knee OA, Spector *et al* reported slightly increased CRP levels, which were predictive of the progression of joint damage.¹⁶ In our group of patients with established OA we found no significant increase in CRP measured by a highly sensitive assay, suggesting that this non-specific marker of inflammation may be raised only in patients with rapidly progressive disease. One of the main limitations of most of the previously developed markers is their lack of tissue specificity. Indeed, S-COMP, which was initially proposed as a cartilage-specific molecule, has subsequently been shown to be synthesised by ligament and synovial fibroblasts.⁵⁶ The same holds true also for YKL-40, which has been shown to be secreted in high amount by chondrocytes and synoviocytes, but is also produced by the liver and is present in several other tissues.⁵⁷ Obviously HA and PIINP are also non-specific for joint tissues, as they are widely distributed and their levels are increased in other conditions, including scleroderma.⁵⁸ The lack of specificity of the current markers is likely to account in part for the discordant and disappointing clinical data generated so far with biochemical markers in joint diseases (for a review see Garnero *et al*).⁵

To overcome this limitation, major efforts have been made to develop more specific markers, especially those reflecting type II collagen degradation, which is a hallmark of OA. Because synovial activity is likely also to have a pivotal role in the pathogenesis of OA, a marker

that specifically reflects its turnover would be useful. Recently, and concomitantly, candidates for these two types of marker have been developed—namely, U-CTX-II and U-Glc-Gal-PYD, for cartilage and synovial tissue, respectively. Because these two assays measure specific cross links of the collagen molecules which constitute the framework of cartilage and synovium matrices, they are likely to reflect specifically the destruction of these tissues. Interestingly, these two new markers were significantly increased in patients with knee OA compared with controls. Of interest, also, is the observation that the urinary levels of these two new biochemical markers are not affected by body weight, which may confound the interpretation of S-PHIINP and S-HA levels in patients with OA.

One of the main potential uses of biochemical markers would be to identify patients at high risk of rapidly progressive joint destruction. Indeed, clinical indices such as pain and physical function score are poorly related to the destruction of joint structure, as confirmed in our study by the absence of a correlation between the total WOMAC index and joint space parameters. Ultimately, the proof of that concept would be obtained from longitudinal studies correlating initial values of biochemical markers to the rate of joint destruction assessed prospectively by repeated radiographic evaluations. To obtain an accurate estimation of the rate of progression of joint destruction in individual patients with knee OA, long term studies will be required because of the low rate of progression in patients with established OA. Although not optimum, cross sectional studies correlating levels of biochemical markers with joint structure indices are likely to give valuable indication of the performance of different markers for that role. In this study we looked at the relations between levels of biochemical markers and a quantitative and continuous measure of joint destruction—that is, JSA and minimal JSW, measured by a state of the art radiographic technique. To our knowledge, this is the first study using this type of analysis. Most previous reports—including the most recent ones^{53–59}—correlating levels of biochemical markers with the degree of joint destruction, were evaluated by a composite index such as the Kellgren and Lawrence grading system. Among the nine different markers investigated, we found that U-CTX-II, S-PHIINP, and U-Glc-Gal-PYD were the most predictive of both JSA and minimal JSW, their levels explaining up to 20% of the interindividual variance of joint space parameters. Interestingly, and as expected from our current knowledge of the pathophysiology of OA, the better predictor of pain and physical function as assessed by the WOMAC index was U-Glc-Gal-PYD, a specific index of synovial tissue activity.

Our study has some limitations. The most important one relates, as discussed above, to its cross sectional design, and thus we could not investigate the value of the markers in predicting progression of joint damage. Also, the number of patients is quite limited. However,

this is probably one of the largest studies including both a panel of recently developed biochemical markers and a quantitative assessment of joint space parameters. We measured only one of the structural features of OA—that is, JSN, and did not evaluate other components, such as osteophytes or subchondral bone sclerosis; however, in both epidemiological studies and clinical trials JSN is considered as the primary end point of OA. We did not measure a marker reflecting specifically the metabolism of aggrecan, the other major protein of the cartilage matrix. Finally, our results are based on a single determination of biochemical markers, which may actually underestimate the true association between these parameters and joint damage because of the variability of the measurements.

In summary, using a panel of biochemical markers of bone, cartilage, and synovium we found that patients with knee OA are characterised by a systemic decreased bone turnover, increased cartilage turnover, and biochemical evidence of synovitis. U-Glc-Gal-PYD, a specific marker of synovial activity, was the most important predictor of pain and physical function. High levels of U-CTX-II, S-PHIINP, and U-Glc-Gal-PYD were associated with increased cartilage loss. In conclusion, assessment of cartilage and synovium metabolism using U-CTX-II, U-Glc-Gal-PYD, and S-PHIINP may be useful for assessing disease activity and joint damage in patients with knee OA, though longitudinal studies are required to confirm that hypothesis.

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